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Lung and Liver Cell-Mediated Mutagenesis Systems Specificities in the Activation of Chemical Carcinogens

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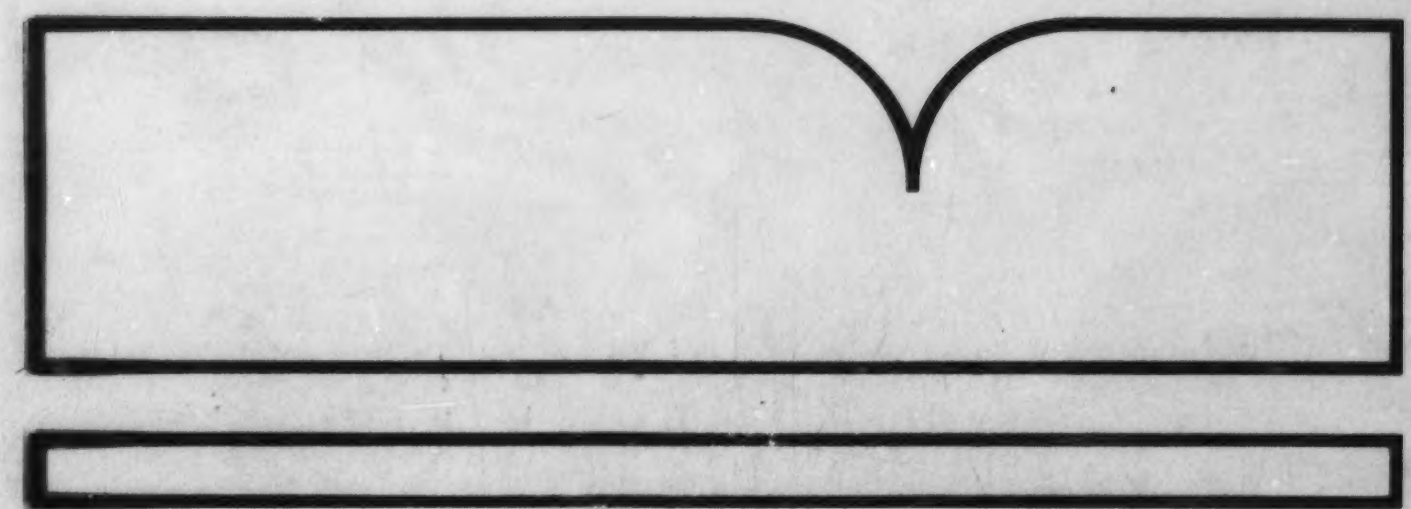

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JOURNAL ARTICLE

Lung and liver cell-mediated mutagenesis systems: specificities in the activation of chemical carcinogens

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Abstract

A liver and lung cell-mediated-V79 cell mutagenesis system using intact cells as metabolic activation systems was employed to study the relative ability of cells from these organs to activate chemical carcinogens. Primary cultures of liver and lung cells from male Sprague Dawley rats were used to metabolically activate the chemicals and the mutation of Chinese hamster V79 cells to ouabain resistance used to detect mutagenic intermediates. 7,12-Dimethylbenz[a]anthracene and 3-methylcholanthrene, were more active in the lung system than in the liver cell system. Benzo[a]pyrene (B[a]P) was inactive in the liver cell-mediated system but mutagenic to V79 cells in the lung cell-mediated system. Dimethylnitrosamine (DMN) was inactive in the presence of lung cells but was mutagenic to V79 cells in the presence of liver cells. Aflatoxin B_1 was mutagenic in the liver cell-mediated system, but only weakly mutagenic in the lung cell-mediated system. Because the mutagenicities of DMN and B[a]P were organ-specific, the metabolism of these carcinogens in the two primary cell systems was investigated. DMN was metabolized by liver but not by lung cells, possibly accounting for its lack of mutagenicity in the lung cell system. B[a]P was extensively metabolized by both cell types, but quantitative differences were observed when the metabolic products were analyzed by high pressure liquid chromatography. Comparing total organic and water soluble metabolites, lung cells produced similar amounts of 7,8- and 9,10-diols but little 4,5-diol, while liver cells produced equivalent total amounts of the three diols. Lung cells produced twice the amount of B[a]P glucuronide conjugates as liver cells, while liver cells produced twice the amount of B[a]P sulfate conjugates as lung. The data suggest that intact cells from various organs can be used as metabolic activating systems in *in vitro* assays and that studies into organ specificity can be investigated by this approach.

Abbreviations: B[a]P, benzo[a]pyrene; DMN, dimethylnitrosamine; DMBA, 7,12-dimethylbenz[a]anthracene; AFB_1, aflatoxin B_1; MCA, 3-methylcholanthrene; h.p.l.c., high pressure liquid chromatography.

Introduction

Many environmental chemical carcinogens are nonreactive and require metabolic activation to manifest biological effects (1,2). Because rapid assays are needed to detect hazardous, nonreactive, environmental agents, and because the target organisms used in these assay systems have metabolic limitations, considerable effort has been devoted to developing metabolic activation systems useful for rapid assays. Preparation of subcellular fractions for assay systems (3–5) may alter the balance of metabolic reactions which exists in intact cells. Indeed, subcellular fractions have been shown to differ from intact cells: (i) in the activation of benzo[a]pyrene (B[a]P)* to intermediate(s) mutagenic to *Salmonella typhimurium* (6); (ii) in the patterns of metabolite profiles (7,8), and (iii) in the formation of DNA adducts (9–11) with B[a]P, DMBA, and aflatoxin B_1 (AFB_1). Furthermore, many chemical carcinogens exhibit an organ specificity in tumor induction (12), and liver homogenates or even intact liver cells used in the assays may not simulate the response of nonhepatic target organs.

We have been interested in developing intact cellular activation systems which may be useful in short term assays as well as in organ specificity studies (13–16). The approach is based on the embryonic fibroblast-mediated mutagenesis system originally developed by Huberman and Sachs (17). By modifying this procedure, intact primary cells from the appropriate tissue can be cocultivated with Chinese hamster V79 cells in the presence of the carcinogen. Mutation of the V79 cells provides evidence that mutagenic intermediates are produced after carcinogen metabolism by the primary cells. Metabolism of the carcinogen may be only one factor in determining organ specificity (12); other factors, such as nature of DNA adducts, repair of DNA damage, or persistence of certain types of DNA adducts, can also be studied with this technique. In the present study, carcinogens from three chemical classes were compared for mutagenic activity in systems containing cocultivated adult rat hepatocytes or adult rat lung cells and V79 cells. The metabolism of dimethylnitrosamine (DMN) and B[a]P was investigated in both liver and lung cells.

Materials and Methods

Liver cell-mediated mutagenesis

Primary liver cells were prepared from male 6–10-wk-old Sprague Dawley rats, as described earlier (13). The liver cells (8×10^6/5 ml, >90% viability by trypan blue dye exclusion) were seeded in complete medium into 25 cm^2 T-flasks containing V79 cells (2.5×10^5; seeded 18 h earlier). The maximum number ($1.5–2.0 \times 10^6$) of attached, viable liver cells was attained 3 h after

seeding, and the medium was then replaced with 8 ml fresh medium containing the chemical of interest. The liver and V79 cells were cocultivated in the presence of the chemical for 48 h prior to being dissociated by 0.25% trypsin/0.02% EDTA in phosphate buffered saline. The V79 cells, distinguishable from the larger liver cells by light microscopy, were counted on a hemocytometer. For cloning efficiency, 200 V79 cells in 5 ml medium were seeded into 60 mm dishes (6 dishes/point), and after 6–7 days, the colonies were fixed and stained with Giemsa. The liver cells did not reseed. The number of mutant colonies was determined by seeding V79 cells (10^5/4 ml medium/60 mm dish, 20 dishes/point), and 2 days later by adding ouabain in 1 ml medium to a final concentration of 1 mM. The mutagenesis experiments were then carried out as previously described (13).

Lung cell-mediated mutagenesis

Primary lung cells were prepared from male 6–10-wk-old male Sprague Dawley rats as described earlier (15). After opening the chest and cervical area to expose the lungs and trachea, a cannula was inserted via the heart into the pulmonary arteries. The lungs were perfused with buffered Hank's balanced salt solution (Gibco, Grand Island, NY) until they became white, and the lungs and trachea were then removed. The isolated lungs were then infused for 10 min via the trachea with an enzyme solution of 0.13% trypsin and 0.01% EDTA, containing 50 U collagenase/ml. During this step, the lungs expanded to approximately 4 times the original volume. The tissue was then minced with scissors (0.5 cm^3 pieces) and further digested in 50 ml of the enzyme solution with gentle stirring for 90 min at 37°C. The supernatant containing individual cells was mixed with 3 ml DNase I (Sigma, St. Louis, MO, 60 U/ml), and the cells were pelleted by centrifugation. The cells were then resuspended in complete medium and seeded at 2 x 10^6 cells/5 ml into 25 cm^2 T-flasks. The viability by trypan blue dye exclusion was >95%, and maximum attachment (~50%) of the epithelial cells occurred by 48 h. The medium was changed, and the cells were maintained an additional 48 h and then irradiated with 4000 R. Only cultures which by light microscopy appeared to have at least 80% epithelial-like cells were used for mutagenesis and metabolism experiments. As previously reported (15), Type I and II epithelial-like cells, macrophages, and endothelial cells have been identified in the primary lung cell cultures. Eighteen hours after irradiation, 5 x 10^5 V79 cells/flask were added in 1 ml medium. After 5 h, the medium was changed to 8 ml fresh medium containing the chemical. Cocultivation lasted 48 h; the cells were then dispersed and reseeded to determine cloning efficiency and number of mutants (13). The V79 cell number was determined by subtracting the number of lung cells per flask (without added V79 cells) from the number of cells in the cocultivated flasks. The irradiated lung cells did not multiply during the cocultivation and did not form colonies on reseeding. Furthermore, initial studies showed that irradiation did not alter B[a]P metabolism profiles or B[a]P's activation to mutagenic intermediates.

Metabolism studies

B[a]P metabolism studies were carried out by adding [^{3}H]B[a]P (4 μM final, specific activity 100 to 350 μCi/μmol, Amersham Corp., Arlington Heights, IL) in 25 μl acetone-methanol (2:1) to the primary cells (liver or lung cells, 1.5–2.0 x 10^6/25 cm^2 T-flask) in 3 ml of medium for 4, 24, or 48 h. After trypsinization, cells were lysed by freeze-thawing. The cells plus medium were extracted with 15 ml ethyl acetate:acetone (2:1) by vortexing for 2 min, followed by centrifuging at 1100 x g for 10 min. Twelve ml of the organic layer was evaporated to dryness with dry filtered N_2. Samples were redissolved in 150 μl methanol:acetone (2:1) containing the appropriate nonradioactive B[a]P metabolite standards, and 10 μl of the solution was injected into a Dupont Model 848 high pressure liquid chromatograph fitted with a 4 mm x 30 cm μ Bondapak C_{18} column (Waters Associates, Inc., Milford, MA) Metabolites were eluted at ambient temperature in methanol:water:ethyl ether (66.3:30.4:3.3, v/v) at a flow rate of 1.8 ml/min. Metabolite identification and quantitation were accomplished by monitoring the effluent by u.v. spectroscopy and collecting the appropriate effluents for liquid scintillation analysis. Retention times have been previously reported (18). The experiment was repeated, with all points in duplicate (two flasks per point), controls without cells were run at all time points, and control values have been subtracted.

After organic extraction, the aqueous layer was analyzed for glucuronides and sulfates. Samples were stored frozen at −80°C until assay, thawed, and re-extracted with ethyl acetate:acetone (2:1). Nitrogen was then bubbled through the aqueous layer for 30 min and the sample volume brought to 10 ml with deionized water. The pH was adjusted to 5.0 with 1 N acetic acid, and the sample was divided evenly. β-glucuronidase (10,000 Fishman units, Sigma Chemical Co.) in 0.1 ml of 0.05 N sodium acetate (pH 5.0) was added to one tube. For sulfatase treatment, 19.2 mg of D-saccharic acid-1,4-lactone was added to inhibit β-glucuronidase, followed by 250 units sulfatase (Sigma, Type VII) in 0.1 ml 0.05 N sodium acetate. The tubes were then incubated for 21 h at 37°C in a shaking water bath. Ethyl acetate:acetone (2:1, 19.5 ml) was then added to each tube, and the organic soluble materials were analyzed as described above. Recoveries in all cases were greater than 85%.

DMN metabolism was determined by adding [^{14}C]-DMN (specific activity 0.67 mCi/mmol, New England Nuclear, Boston,MA) to primary liver or lung cells (10^7) in 5 ml Krebs Ringer buffer (pH 7.4) contained in 25 ml *In Vitro* Reaction Flasks (Kontes, Vineland, NJ). The final DMN concentration was 1 mM. The flasks were gyrated slowly at 37°C for the various times. At 15 min before termination, 0.4 ml of 1 M hyamine hydroxide in methanol was injected into the center well. The

incubation was terminated by injecting 0.2 ml of 30% perchloric acid into the cell suspension, and by 15 min further incubation to allow complete trapping of liberated [^{14}C]CO_2. Recoveries of [^{14}C]bicarbonate were approximately 95%. The center wells were placed in scintillation vials containing Aquasol (New England Nuclear) and counted. Controls containing no cells were run at each time to correct for DMN volatility.

Results

Lung and liver cell-mediated mutagenesis

The data in Table I show the mutagenic activities of carcinogens from three classes of chemicals in the rat liver and lung cell-mediated mutagenesis systems. The noncarcinogenic hydrocarbon, anthracene, was not mutagenic to V79 cells in either the liver or lung cell-mediated systems. DMBA, MCA, and B[a]P at 1 μg/ml were all mutagenic in the lung cell-mediated system, inducing 154, 40, and 59 mutants per 10^6 survivors, respectively. DMBA and MCA were less mutagenic in the liver cell-mediated system than in the lung system, inducing 14 and 8 mutants per 10^6 survivors, respectively. B[a]P, for which rat liver is not considered a target organ, was judged negative in the liver system, inducing only 3 mutants per 10^6 survivors – even at 3 μg/ml B[a]P, a concentration which induced 112 mutants per 10^6 survivors in the lung cell system. DMN, on the other hand, for which rat liver, and not rat lung, is considered a target organ, was mutagenic in the liver cell-mediated mutagenesis system with a dose related response but was not mutagenic in the lung cell system (Table I). AFB_1 induced 43 mutants per 10^6 survivors in the liver cell system but was only weakly mutagenic in the lung cell-mediated system (Table I). No agent reported in Table I was mutagenic to V79 cells in the absence of lung or liver cells (data not shown).

B[a]P and DMN metabolism

Because the target organ specificity of B[a]P and DMN appeared to be reproduced in the liver and lung cell-mediated mutagenesis systems, the metabolism of these carcinogens was studied in the two systems. DMN was metabolized to CO_2 by the liver cells (Table II). However, lung cells, which did not metabolize DMN to intermediates that were mutagenic to V79 cells, also did not metabolize DMN to detectable levels of CO_2. Both cell types remained greater than 80% viable by trypan blue dye exclusion during the assay time. Therefore, the lack of DMN metabolism by lung cells may account for its lack of mutagenicity in the lung cell-mediated system.

The metabolism of B[a]P in the two primary cell types was also studied, and Figure 1 shows the profiles of organic extractable B[a]P metabolites produced by liver and lung cells after 4 h incubation. The major differences in the profiles are that lung cells produce primarily the 7,8-and 9,10-diol with little detectable 4,5-diol, while the liver cells produce the 4,5-diol as the major diol. After 48 h incubation (Figure 1), the lung

Table I

Mutagenic activities of chemicals from three classes of carcinogens in the lung and liver cell-mediated systems[a]

Chemical (μg/ml)	Liver		Lung	
	%CE[b]	Mutants/10^6 survivors	%CE[b]	Mutants/10^6 survivors
Control	78	1 ± 1[c]	72	1 ± 1
Anthracene (3)	71	1 ± 1	70	1 ± 1
DMBA (1)	47	14 ± 7	13	154 ± 42
MCA (1)	62	8 ± 4	28	40 ± 9
B[a]P (1)	58	2 ± 1	24	59 ± 18
B[a]P (3)	52	3 ± 1	16	112 ± 41
DMN (30)	27	63 ± 21	68	2 ± 1
DMN (100)	12	101 ± 34	69	2 ± 1
AFB_1 (3)	21	43 ± 20	62	4 ± 2

[a] See "**Materials and Methods**" for details of liver and lung cell protocols.

[b] CE, cloning efficiency.

[c] Mean ± SD. Comparison between liver and lung data of mutants/10^6 survivors for each chemical was performed by the student t-test. Mutants/10^6 survivors in liver and lung experiments were significantly different at $p = 0.05$ except for anthracene ($p = 0.4$) and AFB_1 ($p = 0.07$).

Table II

Metabolism of DMN to CO_2 by liver and lung cells

Time (h)	[^{14}C]CO_2 formed/10^7 cells (nCi, ± SD[a])	
	Liver cells	Lung cells
1	1.5 ± 0.5	ND[b]
2	4.0 ± 1.5	ND
4	6.5 ± 2.3	ND

[a] SD, standard deviation; the results are the mean ± SD of duplicate determinations from five different cell preparations for each liver and lung. The background was 2.0 ± 0.5 nCi/flask and has been subtracted to determine [^{14}C]CO_2 production.

[b] ND, not detectable over background.

cells produced a large amount of unknown metabolites which migrated in the diol regions. Some of this material was probably the 7,8-and 9,10-diols, but clearly defined peaks were not observed, and much of the radioactive material has not been identified. Therefore, in Table IV this material is listed as "not identified" in the unconjugated B[a]P metabolites. The liver cells at 48 h still produced large amounts of the 9,10-and 4,5-diols with little unidentified radioactive organic soluble material (see Table III).

At 48 h, liver cells had metabolized B[a]P to unconjugated metabolites, which represented 21% of the measured B[a]P metabolite production (Table III). At the same time, 24% of the measured B[a]P metabolite production by lung cells was unconjugated (Table IV).

Table III

Metabolism of B[a]P in rat liver cells[a]

	Dihydrodiols				Quinones			Phenols		
	Pre 9,10	9,10	4,5	7,8	1,6	3,6	6,12	9	3	Total
Unconjugated[b]	1409[c]	270	90	32	32	43	0	17	145	2038 (21%)
Glucuronide-conjugated[d]	1606	202	482	123	205	928	25	45	175	3791 (39%)
Sulfate-conjugated[e]	–[f]	395	224	588	357	2114	39	81	49	3847 (40%)
Total	3015 (31%)	867 (9%)	796 (8%)	743 (7%)	594 (6%)	3085 (31%)	64 (1%)	297 (3%)	369 (4%)	9676 (100%)

[a] Values are mean pmol B[a]P metabolite formed after 48 h of incubation for duplicate samples. Each sample represented 7.5 x 10^5 rat hepatocytes from 3 flasks of treated cells.

[b] Unconjugated metabolites are those extracted from lysed cells and media.

[c] Treatment of the unconjugated metabolites with sulfatase reduced the pre-9,10 diol peak by 63% with concomitant increases in diols, quinones, and phenols. These released metabolites have been incorporated into the sulfate conjugated metabolites.

[d] Glucuronide-conjugated metabolites are those released by treatment of the extracted lysed cells and media with β-glucuronidase.

[e] Sulfate-conjugated metabolites are those released by treatment of the extracted lysed cells and media with sulfatase.

[f] As this material in Figure 2 was β-glucuronidase sensitive, it is not included here, because it is already included in the glucuronide conjugates. (See text.)

Table IV

Metabolism of B[a]P by rat lung cells[a]

	Dihydrodiols				Quinones			Phenols		Not identified	Total
	Pre-9,10	9,10	4,5	7,8	1,6	3,6	6,12	9	3		
Unconjugated[b]	1800[c]	0[d]	0[d]	0[d]	32	45	0	27	60	880	2844 (24%)
Glucuronide conjugated[e]	698	349	72	800	342	1475	78	1883	1437	–	7134 (61%)
Sulfate conjugated[f]	–[g]	360	62	239	173	730	12	147	66	–	1789 (15%)
TOTAL	2498 (21%)	709 (6%)	134 (1%)	1039 (9%)	547 (5%)	2250 (19%)	90 (1%)	2057 (17%)	1563 (13%)	880 (8%)	11767 (100%)

[a] Values are mean pmol B[a]P metabolite formed after 48 h of incubation for duplicate samples. Each sample represented 6.5 x 10^4 rat lung cells from 3 flasks of treated cells.

[d,c] See corresponding footnotes in Table III.

[d] Peaks were not resolved from unidentified metabolites and therefore are included in "Not identified" fraction. (See text)

[e,f,g] See footnotes d, e, and f, respectively, in Table III.

To identify the water soluble metabolites produced by liver and lung cells, the water soluble materials were divided equally and treated with β-glucuronidase and sulfatase. The organic soluble materials extracted after the enzyme treatments were chromatographed, and the profiles are shown in Figure 2. Both liver and lung cells conjugated B[a]P metabolites to glucuronic acid. Total glucuronides represented 39 and 61% of the B[a]P metabolites from liver and lung cells, respectively, (Tables III and IV). β-Glucuronidase released large amounts of pre-9,10-diols after 48 h of B[a]P metabolism by liver cells, whereas lung cells conjugated low levels of these metabolites as glucuronides. Liver cells conjugated more 4,5-diol. Lung cells conjugate high levels of the 3- and 9-phenol to glucuronic acid.

Conjugation of B[a]P metabolites to sulfate was observed in cultures of liver and lung cells, but the metabolite conjugation was somewhat different. Both liver and lung cells produced a major early eluting peak after sulfatase treatment (Figure 2). This peak was demonstrated to be sensitive to β-glucuronidase and therefore is probably a glucuronide of B[a]P which,

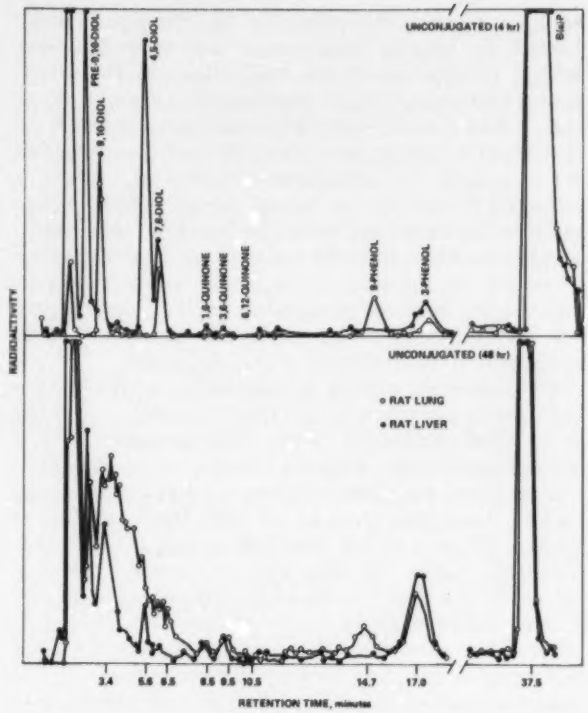

Fig. 1. H.p.l.c. profiles of organic soluble B[a]P metabolites produced by primary rat liver and rat lung cells in culture: upper panel, metabolites produced after 4 h treatment; lower panel, metabolites produced after 48 h treatment. Cells were exposed to 4 μM [^{3}H]B[a]P, and after the stated time of treatment the cells were lysed, and cells and media extracted with ethyl acetate–acetone, and chromatographed on a reverse phase column using an isocratic ternary solvent system. (See **Materials and Methods**).

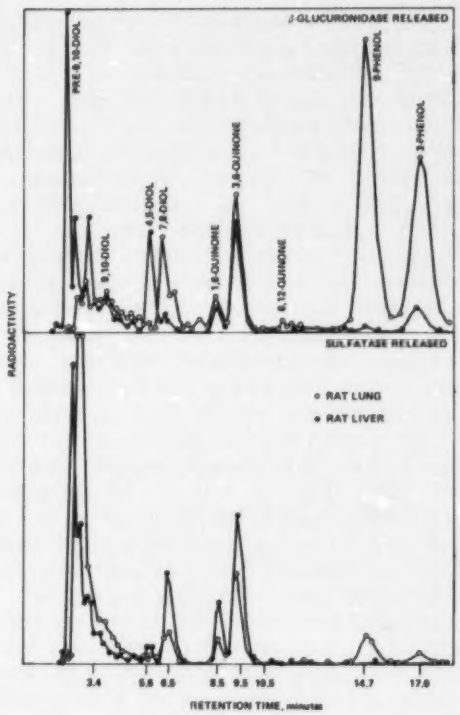

Fig. 2. H.p.l.c. profiles of aqueous soluble B[a]P metabolites produced by rat liver and rat lung cells. Cells were exposed to 4 μM [^{3}H]B[a]P, and after 48 h of treatment the lysed cells and media were extracted with ethyl acetate–acetone. The remaining aqueous layer was treated with β-glucuronidase (upper panel) or sulfatase (lower panel) and the resulting mixture extracted with ethyl acetate–acetone and chromatographed, as described in **Materials and Methods**.

because of the pH required for sulfatase treatment (pH 5), was extracted into the organic phase after sulfatase treatment. The radioactivity from this peak, therefore, has not been included in the pre-9,10-sulfate conjugated metabolites in Tables III and IV. Liver cells conjugated more 4,5-diol to sulfate than lung cells, while lung cells conjugated more 3- and 9-phenols to sulfate.

Samples of both the initial organic extractable metabolites from liver and lung cells were subjected to sulfatase treatment to detect organic soluble sulfate conjugates. It was observed that in liver cells 63% of the pre-9,10-diol peak was reduced with concomitant increase in 9,10-diol, 4,5-diol, 7,8-diol, 1,6-quinone, 3,6-quinone, 9-phenol, and 3-phenol. These values for organic extractable sulfates were added to the sulfatase-released metabolites from the aqueous phase to give the results presented in Table III. Thus, these values appear as "sulfate conjugated" and not "unconjugated" in Table III. Under similar treatment, it was observed that lung cells did not produce significant quantities of organic soluble conjugates.

Discussion

A major concern in developing *in vitro* systems to study the various processes involved in carcinogenesis is how well the *in vitro* results simulate the *in vivo* response. The experiments reported herein indicate that the cell-mediated mutagenesis system may provide a useful approach for *in vitro* study of liver and lung responses to several carcinogens. Many previous *in vitro* studies into liver or lung activation of carcinogens have used subcellular fractions from the tissue for metabolic activation (19–22). However, several reports have indicated that broken cell preparations give different results than intact cells, with intact cells better simulating the *in vivo* situation (7–11). This suggests that the metabolic causes of a chemical's organ specificity may be altered by disruption of the tissue. Furthermore, the use of intact cells allows investigation into other factors which may contribute to organ specificity (12); these factors include the relative uptake of the chemical by different cell types, nature of DNA adducts, and their relative rates of removal in the activating cell and in the target cell.

A possible limitation when using intact cells for activation is whether the active metabolite formed can reach the DNA of the target organism. Data indicate that several classes of carcinogens can be assayed in the cell-mediated system (13,17,23,24,25), but more classes

need to be studied to determine the general applicability of this approach as a short term assay. Another concern about the cell-mediated approach is the possible selection for or against certain cell types. We have previously reported (14) the cell type specificity in the activation of chemical carcinogens which underscores possible effects of selection. In the present work, because primary lung cells required such a long attachment period before being used for the mutagenesis and B[a]P metabolism studies, the possibility of cell selection and/or alteration of enzyme activities exists. However, our previous studies (15) indicated that a mixed population of lung cells were present although certain lung cell populations were not observed. Furthermore, the high level of B[a]P metabolism in the cultured lung cells indicates that these enzymes were not lost in the seeding process. Because of the different attachment times, the comparisons of the lung cells' to the liver cells' ability to activate the carcinogens to mutagens and to metabolize the B[a]P were made at different times in culture (see **Materials and Methods**). While this may interfere with the direct comparison of lung cells to liver cells, it should be emphasized that the metabolism and mutagenesis studies with the cells from the individual tissues were done at the same time and, therefore, are comparable. In the mutagenesis studies with DMN, the lung cells were not used immediately after seeding. Therefore, because the lack of DMN mutagenicity in the lung cell system could be due to the loss of DMN metabolism ability, the metabolism experiments with DMN were initiated immediately after tissue digestion for both liver and lung. As shown in Table II, even at this early time the lung cells could not metabolize DMN at a detectable level, which indicates that the lack of DMN mutagenicity in the lung cell system is probably not due to loss of enzyme activity during seeding. It has also been reported that DMN metabolism was extremely low with rat lung tissue slices (26).

Organ culture, tissue slices, perfused liver and lung, and *in vivo* studies have been previously used to observe differences in carcinogen activation by the two organs (27–35). It has been shown with male Sprague Dawley rats that MCA induced-perfused lung metabolized B[a]P about as efficiently as MCA induced-liver (30), which is in general agreement with our findings of B[a]P metabolism for uninduced liver and lung cells. Kahl *et al.* (32) observed that B[a]P diol-epoxide-DNA adducts were detectable in both liver and lung of MCA-induced mice and concluded that other factors than metabolism and adduct formation must be involved with tissue specificity. Eastman and Bresnick (35) observed that hydrocarbon–DNA adducts persisted longer *in vivo* in lung than in liver and that the strain of mouse with the highest level of prolonged binding had a higher susceptibility to neoplastic transformation. Boroujerdi *et al.* (34) reported that in male Sprague-Dawley rats the B[a]P-diol epoxide DNA adduct was not found in rat liver and only a relatively small amount was observed in rat lung. These authors observed that the major adduct formed *in vivo* in both tissues was a B[a]P-phenol adduct as opposed to the B[a]P-diol epoxide adduct found in more susceptible species such as the A/J mouse (34). Ashurst and Cohen (27) found that the B[a]P-7,8 diol-epoxide formed the major DNA adduct in isolated MCA-induced or uninduced hepatocytes, which is different from what we would predict based on our metabolism studies and what was found by Jernstrom *et al.* (33) for MCA-induced hepatocytes. Further studies to resolve the apparent discrepancies in DNA adducts between the different approaches, systems, and species will lead to a better understanding of organ and species specificity in the initiation of carcinogenesis.

The mutagenic activity of metabolites of B[a]P have been previously investigated. B[a]P 7,8-diol is reported to be highly mutagenic in the fibroblast-cell-mediated mutagenesis system, while the 4,5-diol was inactive (25). Furthermore, the 7,8-diol-9,10-epoxide of B[a]P has the highest mutagenic activity of any B[a]P derivative studied (25,36). In the lung cell system, the 7,8-diol (compare Tables III and IV), a precursor of the mutagenic 7,8-diol-9,10-epoxide is formed to a slightly greater extent than in the liver cells; however, this observation alone probably cannot explain the large difference in mutation between the two systems. It has been reported by Jones *et al.* (37) that rat hepatocytes produce quantities of B[a]P-3-sulfate, a conjugate which is soluble in organic solvents. Cohen and Moore (38) have reported an early eluting metabolite appearing in organic extracts from lung organ cultures. In the present study we observed that 63% of the organic soluble pre-9,10-diol metabolites from liver cells was converted to diols, quinones, and phenols by sulfatase treatment. No such effect was seen with organic soluble metabolites formed by lung cell cultures. Thus, differences in the formation of early eluting organic soluble metabolites exist between liver and lung cell cultures.

A tissue specificity for metabolism of B[a]P at specific ring positions was observed. Lung cells metabolize 23% of the B[a]P metabolized at ring positions 9,10; while liver cells metabolize only 12% (Table III and IV). Both cell types metabolized B[a]P to 3,6 position metabolites to about the same extent. Liver cells are much more specific for 4,5 position metabolism than lung cells (8% *vs.* 1%). Liver cells seemed to be more efficient at conjugating B[a]P-3,6-quinone to sulfate conjugates, while lung cells conjugated this metabolite to glucuronic acid more readily. Lung cells also produced greater B[a]P-phenols than did liver cells, representing 30% of the total B[a]P metabolized.

In the present study the metabolism of two carcinogens, B[a]P and DMN, was investigated to gain insight into the causes of their target organ specificity. For DMN, the lack of metabolic activation by rat lung cells apparently accounts for its lack of mutagenicity in the lung cell-mediated system. Although B[a]P could be metabolized by either the liver or lung cell system, both

qualitative and quantitative differences in the metabolites existed.

Our studies on the metabolism of B[a]P in lung and liver cell cultures do not seem to reveal a marked difference which could explain with certainty the differences in mutation responses. It is likely that either other uncharacterized metabolites or other processes leading to mutation might play a major role in the organ specific induced mutation response to B[a]P.

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